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Prepared by
THE DUDLEY OBSERVATORY
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The Microbiological Flora of the Gemini IX Spacecraft Before and After Flight

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INTRODUCTION

In the course of microbiological exposure and collection experiments (1) on the Gemini IX spacecraft, it appeared desirable to establish the number and type of microorganism within the spacecraft immediately before and after flight. For this purpose three locations were chosen which were representative of frequently touched, occasionally touched, and protected or dust collecting locations. These were swabbed at suitable times, and the swabs were returned to the laboratory for examination.

MATERIALS AND METHODS

Swabbing Technic: Falcon "swube" disposable swabs were used for the swabbing of the selected areas within the capsule. The swabs were dispatched to Cape Kennedy prior to the start of the spacecraft, after the technicians preparing the spacecraft for launch had been instructed in the technic of swabbing. Dry swabs were used for the swabbing of three to four locations in the space capsule. Four sets of swabs were obtained, called "Preflight set No. 1", "GT-9A prescrub" set, and "preflight set No. 2", which were all taken before the flight, and one "postflight" set. The preflight set No. 1 swabs were taken on May 16, and the GT-9A prescrub set swabs on May 31, 1966.

These samples resulted from delays in the actual flight schedule and were originally intended to be preflight swabs. Preflight set No. 2 swabs were taken on June 3 before the start of the spacecraft, and the postflight set swabs were taken on June 6 after the flight. The swabbing locations (see Figs. 1 and 2) of the first set were [1] - R/H door handle; [2] - above center panel L/H side; and [3] - behind R/H seat. After being returned to the New York State Division of Laboratories and Research at Albany, the swabs were stored at 4° C before they were eluted.

Bacterial Diagnostic Technics

Elution Procedure: On inspection, the swabs were found to be heavily loaded with dirt (see Fig. 3). They were each immersed and squeezed out in 5 ml of thioglycollate broth, then eluted for 60 min at 4° C and subsequently agitated on a mixer. Samples of all eluted suspensions were then distributed among three groups (group 1, 2, and 3) of investigators who obtained their results completely independently.

Group 1: 0.5 to 1 ml quantities of the eluted suspensions were diluted in 9 to 9.5 ml of thioglycollate broth, then incubated at 37° C. The suspension cultures were checked for bacterial growth after 24 and 48 hours of incubation, and blood agar plates were inoculated with the turbid suspensions and incubated under aerobic and anaerobic conditions. The organisms isolated were characterized by group 2. In addition, 0.5 ml aliquots of the eluted suspensions were diluted in 4 ml each of an 0.6 g % agar suspension, and 1.5 ml aliquots of the mixture were poured on a

Sabouraud agar plate which was incubated for 3 days at room temperature and 2 blood agar plates which were incubated for 2 days at 37° C, one under aerobic and one under anaerobic conditions.

Group 2: The eluted suspensions investigated by group 2 were studied by the methods of determinative bacteriology (2). Apart from morphological studies, the isolates were tested for hemolysis, starch hydrolysis (3), production of acetylmethylcarbinol from dextrose (3), gelatin hydrolysis (3), growth in thioglycollate (Difco) broth over an observation period of 21 days, growth in litmus milk (Difco) and Pennassay broth (Difco), H_2S (3) and indole production (3), nitrate reduction (4), ureae hydrolysis (5), fermentation of glucose, maltose, xylose, sucrose, mannitol, lactose and galactose were also studied.

Methods used by group 3 included incubation at room temperature on trypticase soy (BBL)[1] blood agar for 19 days and on Sabouraud dextrose agar (BBL) for 26 days, and suspensions in thioglycollate medium (BBL) were incubated for 50 days. The identifications of all growth forms discovered were based on standard methods of determinative bacteriology (2).

RESULTS

The results are given in Tables 1 and 2. The group 1 blood agar plates show that the preflight set No. 1, swabs Nos. 1 and 2, the preflight set No. 2, swabs Nos. 1 and 3, the GT-9A prescrub set, swabs Nos. 2 and 3, and the postflight set, swab No. 4, had picked up numbers of microorganisms which varied between approximately 20 to 140 per swab (see Table 1).

[1] Baltimore Biological Laboratory, Inc., P.O. Box 6711, Baltimore, Maryland 21204.

The Sabouraud plates of preflight set No. 1, swabs Nos. 1 and 2, showed growth of a Penicillium species, and an unidentified fungus was found on GT-9A prescrub set, swab. No. 3. The thioglycollate broth suspensions showed growth of Micrococcus ureae, Bacillus cereus and Bacillus megaterium of preflight set No. 1, swabs Nos. 1 and 2 (see Table 2). The thioglycollate broth results, in contrast to the agar plate results, did not show any growth of the preflight set No. 2, swab No. 1, the GT-9A prescrub set, swab No. 3, and the postflight set, swab No. 4.

The results of group 2 include long-term observations of the cultivated suspensions up to 21 days as well as diagnostic studies of the microbial growth forms found. After longer incubation periods, the primary thioglycollate suspensions showed growth of microorganisms which were identified as a Penicillium species in the preflight set No. 1, swab No. 3, as B. megaterium in the GT-9A prescrub set, swab No. 3, as B. pumilis in the postflight set, swab No. 3, and as an Aspergillus species in the postflight set, swab No. 4 (see Table 2). During the further studies, hemolytic B. cereus and Micrococcus ureae were found on preflight set No. 1, swab No. 1, which agrees with the results of group 1. B. subtilis was isolated from preflight set No. 1, swab No. 2, of which a Penicillium species, B. megaterium and B. cereus were found by group 1. Corynebacterium acne was found on preflight set No. 2, swab No. 2. Swab No. 3 of this set showed B. subtilis and Micrococcus ureae. The corresponding group 1 result showed microbial growth under conditions

of aerobic and anaerobic incubation which was not further identified. No growth was found in preflight set No. 2, swab No. 1, in GT-9A prescrub set, swabs Nos. 1 and 2, and in postflight set, swabs Nos. 1 and 2. In comparison, preflight set No. 2, swab No. 1, and GT-9A prescrub set, swab No. 2, showed growth of microorganisms in the results of group 1.

Group 3. Analogous to the results of groups 1 and 2, preflight set No. 1, swabs Nos. 1 and 2 were found contaminated (see Tables 1 and 2) and Staphylococcus albus and B. cereus were isolated from swab No. 1, and B. subtilis from swab No. 2 (see Table 2). Flavobacterium breve and and a Streptococcus species were isolated from preflight set No. 2, swab No. 3. This swab also showed contamination of the corresponding swabbed area in the results of groups 1 and 2. The GT-9A prescrub set, swab No. 2, showed contamination of the swabbed area with B. subtilis. The results of group 1 also showed growth of this swab whereas no microorganisms were isolated by group 2. B. coagulans was isolated of postflight set, swab No. 3, of which B. pumilis was isolated by group 2, whereas no growth was found by group 1. No growth was found of preflight set No. 1, swab No. 3, preflight set No. 2, swabs Nos. 1 and 2, of GT-9A prescrub set, swabs Nos. 1 and 3 and of swabs Nos. 1, 2 and 4 of the postflight set.

In comparison, a Penicillium species was isolated by group 2 of preflight set No. 1, swab No. 3, and no growth of this sample was found by group 1. Bacterial growth was found by group 1 of preflight set No. 2, swab No. 1, whereas the results of groups 2 and 3 show no growth.

Preflight set No. 2, swab No. 2, showed contamination of the swabbed area with Corynebacterium acne according to group 2, whereas the group 1 and 3 results showed no growth. No microbial growth at all was found by groups 1, 2, and 3 of the GT-9A prescrub set, swab No. 1, and of the postflight set, swabs Nos. 1 and 2. The GT-9A prescrub set, swab No. 3, showed microbiological growth in the results of group 1, and B. megaterium was identified by group 2 of this swab in contrast to the group 3 result. Whereas the group 1 result did not show any growth of the postflight set, swab No. 3, B. pumilis was isolated from this swab by group 2 and B. coagulans by group 3. In contrast to the group 3 result, bacterial growth was found on postflight set, swab No. 4, by group 1, and an Aspergillus species was identified by group 2.

CONCLUSIONS

While the detailed results described here, because of the obvious variation, demand careful interpretation, this study clearly shows that the Gemini IX spacecraft, as would be expected, was contaminated with an assortment of microorganisms before and after the space journey in the locations tested by swabbing. As shown in Fig. 3 most of the swabs were heavily loaded with dust. The differing spectra of microorganisms found by the three examining groups is of interest in view of the different approaches used. Group 1, a biological research laboratory group, merely attempted a quantitative assay with little identification. Group 2 also was a biological research laboratory group, and group 3 a hospital diagnostic laboratory

group. It is evident that groups 2 and 3 found different types of organisms, the differences presumably having been determined by the choice of methods. This shows that one is likely to find only those organisms for which one is specifically equipped to look. These results also bear on the need for spacecraft sterilization if existing strictures (6, 7) regarding contamination by terrestrial microorganisms are to be implemented.

SUMMARY

Three sites within the Gemini IX space capsule were investigated for microbiological contamination by swabbing before and after the flight. Bacterial or mold growth was observed in three sets of swabs taken before the flight and one set of swabs taken after the flight, and most of the swabs were then found heavily covered with dust. The results were obtained by the completely independent study of the eluted suspensions of the swabs by three research groups and, despite the considerable variation of the detailed results, they show that the inside of the Gemini IX space capsule was contaminated with microbiological materials both before and after the flight.

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Table 1

Number of Microorganism Eluted from Swab Used for Sampling Different Areas of the Gemini IX Spacecraft

Swab Set	Swab No.	No. of Colonies* (Group 1)	Results of**	
			Group 2	Group 3
Preflight Set No. 1	1	69	+	+
	2	138	+	+
	3	0	+	0
GT-9A Prescrub Set	1	0	0	0
	2	23	0	+
	3	46	+	0
Preflight Set No. 2	1	17	0	0
	2	0	+	0
	3	68	+	+
Postflight Set	1	0	0	0
	2	0	0	0
	3	0	+	+
	4	68	+	0

*Group 1 results after plating suspensions eluted from the swabs on blood and Sabouraud agar. The blood agar plates were incubated aerobically or anaerobically at 36° C.; the Sabouraud agar plates were aerobically incubated at room temperature of approximately 22° C. The figures were obtained by multiplying the colonies counted per swab by the dilution factors.

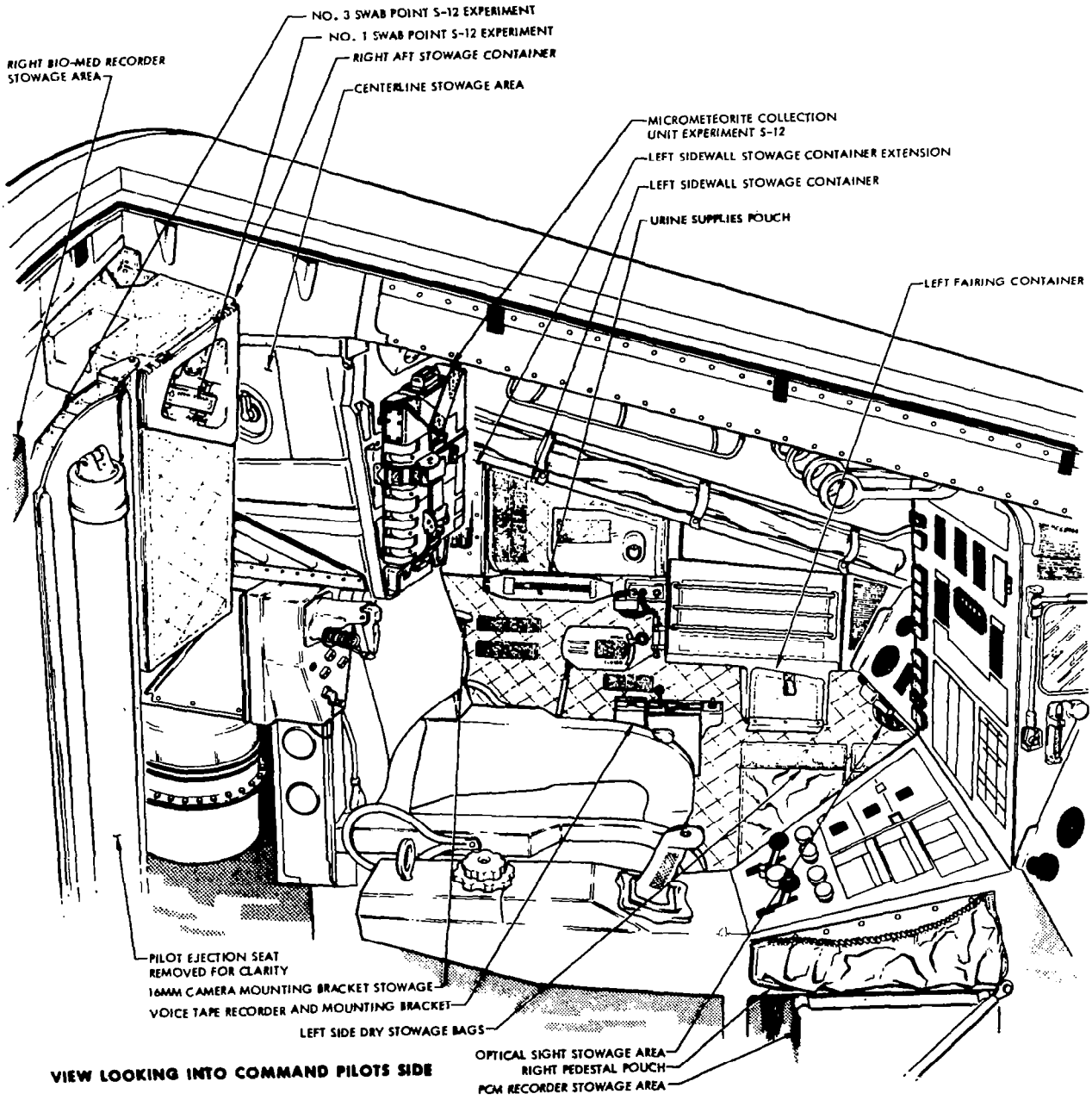
**The group 2 and 3 results, which did not include a quantitative assay, are mentioned for comparison; + = positive growth, 0 = no growth.

Table 2

Microorganisms on the Gemini IX Space Capsule Before and After the Flight

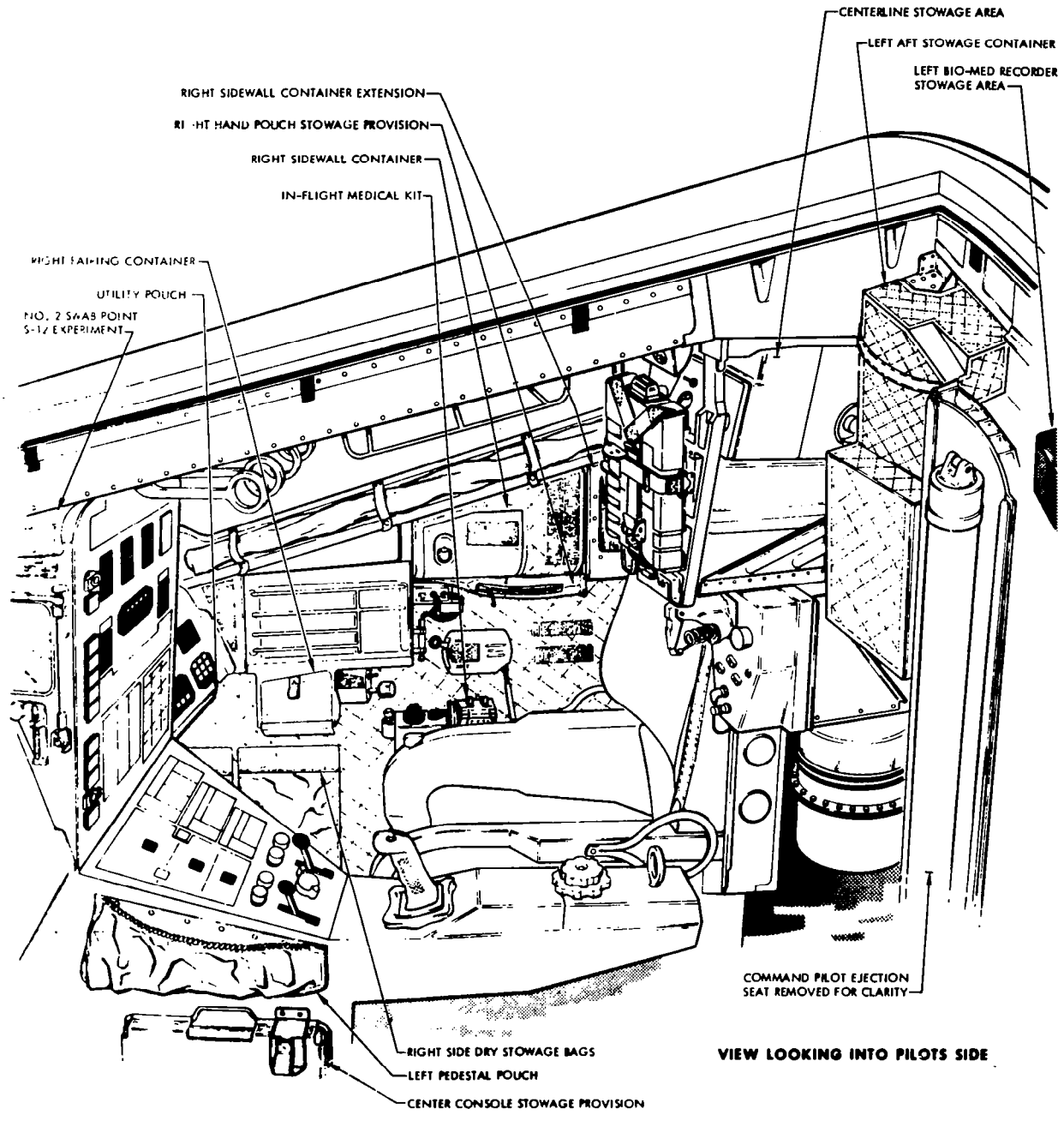
Swab Set	Swab No.	Species of Microorganism		
		Group 1	Group 2	Group 3
Preflight Set No. 1	1	M. ureae, B. cereus Penicillium	B. cereus, M. ureae	S. albus, B. cereus
	2	B. megaterium B. cereus, Penicillium	B. subtilis	B. subtilis
	3	0	Penicillium	0
GT-9A Prescrub Set	1	0	0	0
	2	+*	0	B. subtilis
	3	Fungus*	B. megaterium	0
Preflight Set No. 2	1	+	0	0
	2	0	Corynebacterium acne	0
	3	+	B. subtilis, M. ureae	Flavobacterium breve Streptococcus species
Postflight Set	1	0	0	0
	2	0	0	0
	3	0	B. pumilis	B. coagulans
	4	+	Aspergillus	0

*Unidentified



Spacecraft Interior Stowage Areas (Sheet 1 of 2)

Figure 1.- Swabbing location of swabs Nos. 1 and 3 in a diagram of the interior of Gemini IX spacecraft.



Spacecraft Interior Stowage Areas (Sheet 2 of 2)

Figure 2.- Swabbing location of swab No. 2 in a diagram of the interior of Gemini IX spacecraft; this location is seen from the side opposite to that of Figure 1.

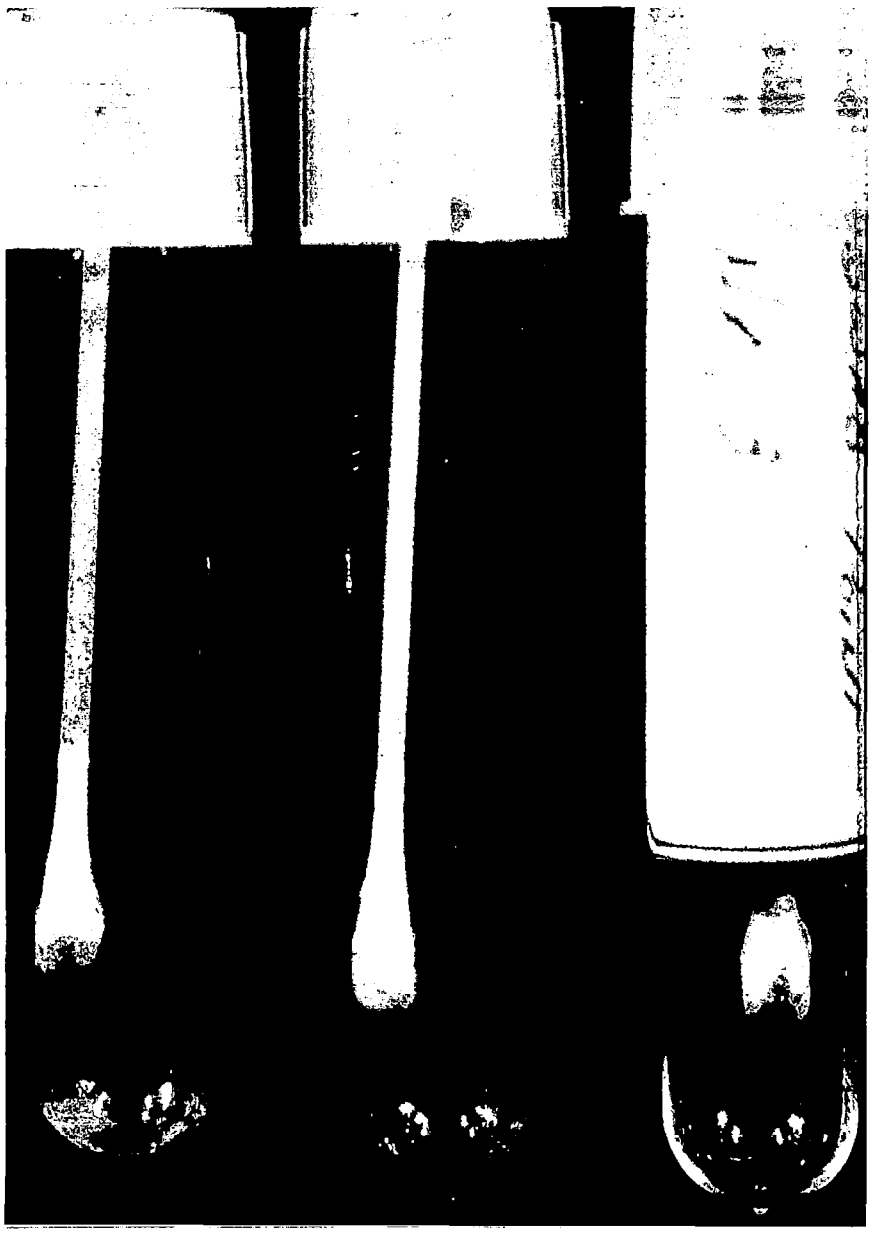


Figure 3.- Swabs representing the GT-9A prescrub set (left swab), preflight set No. 1 (center swab), and postflight set (right swab).